Miniseries on promising pharmacological targets for NASH
NAFLD and Alcohol-Related Liver Diseases

JOURNAL
OF HEPATOLOGY

FXR agonists in NASH treatment

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Summary

The farnesoid X receptor (FXR), a bile acid (BA)-activated nuclear receptor highly expressed in the liver and intestine, regulates the expression of genes involved in cholesterol and bile acid homeostasis, hepatic gluconeogenesis, lipogenesis, inflammation and fibrosis, in addition to controlling intestinal barrier integrity, preventing bacterial translocation and maintaining gut microbiota eubiosis. Non-alcoholic steatohepatitis (NASH), an advanced stage of non-alcoholic fatty liver disease, is characterized by hepatic steatosis, hepatocyte damage (ballooning) and inflammation, leading to fibrosis, cirrhosis and hepatocellular carcinoma. NASH represents a major unmet medical need, but no pharmacological treatments have yet been approved. The pleiotropic mechanisms involved in NASH development offer a range of therapeutic opportunities and among them FXR activation has emerged as an established pharmacological target. Various FXR agonists with different physicochemical properties, which can be broadly classified as BA derivatives, non-BA-derived steroidal FXR agonists, non-steroidal FXR agonists, and partial FXR agonists, are in advanced clinical development. In this review we will summarize key preclinical and clinical features of the most advanced FXR agonists and critically evaluate their potential in NASH treatment.

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NASH, a major unmet medical need

Non-alcoholic fatty liver disease (NAFLD), a chronic disease characterized by lipid accumulation in the liver, can be considered the hepatic manifestation of the metabolic syndrome, a cluster of interrelated clinical features, including insulin resistance with elevated fasting glycaemia, dyslipidaemia, hypertension, and visceral obesity.[1] NAFLD is currently the most common chronic liver disease, globally affecting about 30% of the adult population. Of patients with NAFLD, about a quarter will develop non-alcoholic steatohepatitis (NASH),[2] an aggressive variant characterized by hepatocyte damage (ballooning), and inflammation, leading to liver fibrosis, cirrhosis and hepatocellular carcinoma.[3] NASH has become the second leading indication for liver transplantation and its incidence is further rising.[4]

Despite its alarming prevalence and urgent medical need, no drugs have yet been approved for the treatment of NASH. The complexity of NASH pathophysiology offers multiple potential targets for intervention,[5] and among them the farnesoid X receptor (FXR) is considered a promising one.

FXR: an established pharmacological target in NASH

FXR is a bile acid (BA)-activated nuclear transcription factor expressed at elevated levels in the liver, ileum, kidney, and adrenal glands, and at lower levels in several other tissues.[6] A seminal discovery in 1999 demonstrated that BAs are the endogenous FXR agonists, showing their capacity to activate FXR at physiological concentrations.[6–8] In addition to the nuclear receptor FXR, the membrane receptor Takeda G protein-coupled receptor 5 (TGR5) is also activated by BAs[9] and regulates multiple signalling pathways, maintaining glucose homeostasis, reducing hepatic steatosis, and promoting anti-inflammatory responses.[10]

The breadth and complexity of FXR actions are remarkable, as FXR regulates over 300 primary response genes directly and likely thousands more genes indirectly by binding either as a monomer, homodimer, or as a heterodimer with the retinoid X receptor to a variety of FXR response elements on target genes, thus regulating multiple metabolic pathways.[11–13] FXR controls BA synthesis,[14] transport and enterohepatic circulation[15] and is also a key regulator of pathways in the liver and intestine, influencing lipid[16] and glucose[17] metabolism. FXR activation has a marked capacity to inhibit innate and adaptive immune responses, reviewed in,[18] and fibrosis.[19,20] In addition, FXR transcriptionally regulates genes controlling coagulation,[21] vascular remodelling[22,23] and antibacterial activity,[24] protects the intestinal barrier[25–27] and modulates the gut microbiota.[28]

Thus, FXR controls multiple pathogenetic pathways relevant to NASH and its activation has been shown not only to effectively inhibit NASH development, but also to revert its consequences, in particular liver fibrosis.[29]

Keywords: Farnesoid X receptor agonists; non-alcoholic steatohepatitis; liver fibrosis; randomized controlled trials; combination therapy.
Received 14 March 2023; received in revised form 19 June 2023; accepted 16 July 2023; available online 9 August 2023

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https://doi.org/10.1016/j.jhep.2023.07.034

Keypoints

- The farnesoid X receptor (FXR) controls multiple pathogenetic pathways in non-alcoholic steatohepatitis (NASH), regulating bile acid synthesis and enterohepatic circulation, lipid and glucose metabolism, inflammation, fibrosis, gut barrier integrity and intestinal microbiota.
- FXR, due to its pleiotropic effects in NASH, is an established pharmacological target in this indication, as demonstrated by the variety of FXR agonists in phase II/III clinical trials.
- FXR agonists in advanced clinical trials include bile acid derivatives, non-bile acid-derived steroidal FXR agonists, non-steroidal FXR agonists, and partial FXR agonists.
- Pruritus induction, low-density/high-density lipoprotein imbalances, and modest alkaline phosphatase increases are bona fide class-specific effects of FXR agonists.
- FXR agonists have potential in NASH therapy, based on their clinically proven effects on fibrosis, in combination treatments if not as monotherapy.

Effects of FXR activation in NASH

Building on the original observation of elevated serum BAs, increased serum and hepatic cholesterol and triglycerides, and a pro-atherogenic serum lipoprotein profile in FXR knockout mice,[15] FXR deficiency has been shown to lead to the development of NASH in a mouse model of hypercholesterolemia, indicating a key role of FXR in NASH.[30] Indeed, multiple pathogenetic pathways inducing and sustaining NASH development centre around FXR and can be modulated by FXR activation to inhibit NASH (Fig. 1).

A key feature of NAFLD is the aberrant intrahepatic triglyceride (TG) accumulation, originating primarily from an imbalance between *de novo* lipogenesis and fatty acid (FA) uptake, and their disposal by oxidization or export into the systemic circulation as constituents of very low-density lipoproteins.[1] FXR plays a primary role in lipid regulation, as demonstrated by the significantly increased serum and hepatic TG, cholesterol, and free fatty acid (FFA) levels observed in FXR-deficient mice.[15] In hepatocytes, FXR activation suppresses the expression of sterol regulatory element factor binding protein-1c (SREBP-1c), a critical transcription factor that regulates hepatic TG synthesis by inducing key enzymes involved in lipogenesis, such as FA synthase.[16] The inhibition of SREBP-1c expression by FXR is mediated by a signalling cascade involving the orphan nuclear receptor small heterodimer partner (SHP), which inhibits the capacity of liver X receptor (LXR) to induce SREBP-1c expression by interacting with liver receptor homologue 1.[31] In addition to suppressing *de novo* lipogenesis, FXR activation, in human but not in mouse cells, induces expression of peroxisome proliferator-activated receptor (PPAR) α and its target genes to promote FFA oxidation.[32] Several other FXR target genes contribute to the elimination of serum lipoproteins, including scavenger receptor class B type I (SR-BI), which is critical for reverse cholesterol transport via the transport of high-density lipoprotein (HDL) into the liver,[33] and apolipoprotein C-II, which promotes TG hydrolysis.[34] Thus, FXR activation reduces liver steatosis and hypertriglyceridaemia by suppressing *de novo* lipogenesis and promoting TG oxidation and clearance (Fig. 1).

In ileal enterocytes, FXR activation upregulates expression of fibroblast growth factor (FGF)19 (FGF15 in rodents), a hormone that binds to the FGF receptor 4 (FGFR4)/β-Klotho receptor complex in hepatocytes, inhibiting BA synthesis by downregulating the rate-limiting enzyme CYP7A1,[35] which can also be inhibited via SHP in the liver.[36] However, activation of hepatic FXR appears to be able to suppress BA synthesis via induction of SHP independently of FGF19.[37]

FXR induces another member of the FGF family, FGF21,[38] which is expressed in hepatocytes and acts in the central nervous system and adipose tissue to control glucose, lipid, and energy metabolism. Both FGF19 and FGF21 analogues have been shown to decrease steatosis, inflammation, and fibrosis in various NASH models and in clinical studies, highlighting their relevance in NASH and their potential as therapeutic agents.[39]

FXR controls glucose metabolism through regulation of gluconeogenesis and glycogenolysis in the liver, and regulation of peripheral insulin sensitivity in striated muscle and adipose tissue.[17] FXR-deficient mice show elevated serum glucose and impaired glucose and insulin tolerance, with reduced inhibition of hepatic glucose production by insulin and reduced peripheral glucose disposal.[17] A close relationship exists between insulin resistance and NAFLD, and insulin resistance is implicated both in the pathogenesis of NAFLD and in the progression to NASH.[40] FXR activation in patients with type 2 diabetes and NASH increases insulin sensitivity and reduces serum markers of liver inflammation and fibrosis,[41] supporting potential beneficial actions of FXR agonists in glycaemic control.

FXR signalling is involved in both innate and adaptive immune responses associated with chronic inflammation in NASH, and FXR activation has been shown to inhibit nuclear factor κ-light-chain enhancer of activated B cells (NF-κB),[42] a key transcription factor in inflammation, thus inhibiting neutrophil accumulation and pro-inflammatory mediators, including cytokines like IFNγ and TNFα, inducible nitric oxide synthase, and cyclooxygenase-2.[18] FXR activation has also been shown to downregulate expression of monocyte chemoattractant protein 1 (MCP-1, also known as CCL-2),[43] a key chemokine implicated in the induction and progression of NASH.[44] Notably, hepatic expression of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome increases during the development of experimental and clinical NASH and FXR activation inhibits NLRP3 expression, reducing hepatocyte injury, liver inflammation and fibrosis.[45]

Fibrosis is the result of excessive production of extracellular matrix (ECM) not adequately balanced by its degradation. In the liver, hepatic stellate cells (HSCs) constitute the dominant ECM-producing fibroblasts, contributing approximately 80% to

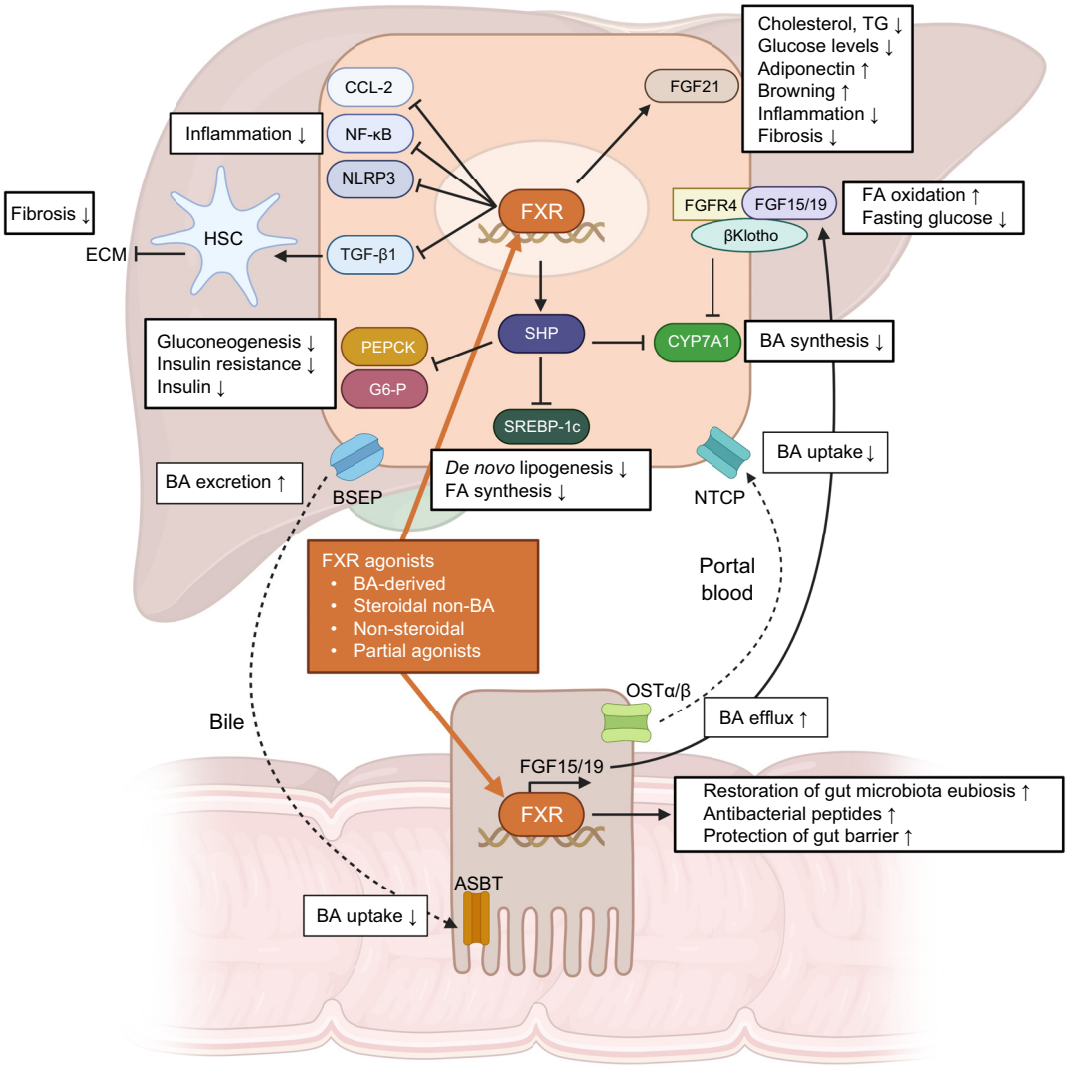

Fig. 1. Effects of FXR signalling in NASH. BAs produced intrahepatically from cholesterol are actively excreted via BSEP into bile. Enterocytic uptake and basolateral export to portal blood are mediated by ASBT and OSTα/β, respectively, and the enterohepatic circulation is closed by hepatocellular BA reuptake from portal blood via NTCP. FXR activation increases biliary secretion (via BSEP) and reduces BA synthesis and BA uptake (via NTCP), maintaining their homeostasis. The systemic effects of hepatic FXR activation in NASH include upregulation of FA oxidation, decreased *de novo* lipogenesis and FA synthesis via SHP-mediated inhibition of SREBP-1c, as well as downregulated gluconeogenesis, contributing to reduced insulin resistance. In addition, FXR activation leads to reduced inflammation, by inhibiting NF-κB, NLRP3, and CCL-2, and to reduced fibrosis by inhibiting TGF-β1 secretion and ECM deposition by HSCs. Activation of hepatic FXR also upregulates production of FGF21, leading to reduced cholesterol and triglyceride synthesis, decreased glucose levels, increased adiponectin and adipocyte browning, while contributing to inhibit liver inflammation and fibrosis. Intestinal FXR activation leads to elevated FGF15/19 levels which bind to FGFR4/β-Klotho and mediate increased FA oxidation, decreased fasting glucose via increased glycogen synthesis, and decreased BA synthesis by inhibiting CYP7A1. CYP7A1 can also be inhibited by hepatic FXR activation via SHP. In addition, FXR activation protects the integrity of the intestinal barrier, preventing bacterial translocation and inducing antibacterial peptides, and restores gut microbiota eubiosis. FXR agonists clinically tested in NASH include BA-derived compounds (OCA), steroidal non-BA-derived FXR agonists (EDP-305), non-steroidal FXR agonists (PX-104, cilofexor, TERN-101, tropifexor, MET-409, vonafexor) and partial FXR agonists (nidufexor). ASBT, apical sodium-dependent bile acid transporter; BA, bile acid; BSEP, bile salt export pump; CCL-2, C–C motif chemokine ligand 2; ECM, extracellular matrix; FA, fatty acid; FGF, fibroblast growth factor; FXR, farnesoid X receptor; HSC, hepatic stellate cell; G6-P, glucose-6-phosphate; NASH, non-alcoholic steatohepatitis; NF-κB, nuclear factor κ-light-chain enhancer of activated B cells; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; NTCP, sodium taurocholate cotransporting polypeptide; OCA, obeticholic acid; OSTα/β, organic solute transporter-α/β; PEPCK, phosphoenolpyruvate carboxykinase; SHP, small heterodimer partner; SREBP-1c, sterol regulatory element factor binding protein-1c; TG, triglyceride; TGF-β1, transforming growth factor-β1.

95% of collagen-producing myofibroblasts in different mouse models of fibrosis, including NASH.[46] HSCs transformed into myofibroblasts deposit collagen I and fibronectin in the ECM and eventually produce fibrogenic cytokines like TGF-β1.[47] In addition, hepatocyte steatosis and injury as well as inflammatory mediators activate a multicellular network including macrophages, natural killer cells, natural killer T cells, and B cells that control HSC activation and fibrosis development.[48] The magnitude of liver fibrosis is the main determinant of long-term outcomes in patients with NASH,[49] and is thus a key therapeutic target. Therefore, any pharmacological treatment in NASH requires robust antifibrotic activity. FXR is expressed at low levels in HSCs and it is unclear if it directly regulates their activation.[50,51] Nevertheless, possibly via an anti-inflammatory effect on HSCs, FXR activation has been shown to prevent liver fibrosis progression, reverse fibrosis and cirrhosis development, and to significantly decrease portal hypertension in different models of NASH.[20,52–54]

FXR signalling along the gut-liver axis affects BA synthesis and enterohepatic circulation, intestinal barrier integrity and bacterial translocation, and modulates the gut microbiota,[55] which plays an important role in the pathogenesis of NASH. As NASH progresses, gut microbial communities become less diverse and exhibit a higher abundance of Streptococcus and Gram-negative bacteria.[3] The altered microbiota disrupt the intestinal barrier, targeting mucus lining, intercellular junctions on the epithelial layer, and the recently characterized endothelial gut vascular barrier (GVB).[26] Microbiota-driven GVB disruption is a prerequisite for NASH development, as shown in animal models, and FXR activation prevents and treats GVB disruption caused by dysbiosis, decreasing bacterial translocation to the liver and thereby reducing inflammatory drivers.[26] Changes in BA composition induced by FXR activation directly and via FGF15/19 also restore the decreased intestinal microflora diversity caused by a high-fat diet and contribute to NASH treatment.[56] In addition, FXR activation increases ileal expression of antimicrobial peptides angiogenin-1 and alpha-5-defensin, which are reduced in cirrhotic rats.[57]

An overview of FXR-mediated effects relevant to NASH is provided in Fig. 1.

Diversity of FXR agonists in advanced development for NASH treatment

BAs, the endogenous FXR agonists, are steroid end-products of cholesterol catabolism and can be categorized into primary BAs (chenodeoxycholic acid [CDCA] and cholic acid [CA]), synthesized from cholesterol in the liver and secondary BAs (deoxycholic acid [DCA] and lithocholic acid [LCA]), produced by intestinal bacteria from primary BAs. CDCA is the most potent endogenous human FXR agonist (EC50 = 10 μM), followed by LCA and DCA, whereas CA does not activate FXR.[7] The identification of endogenous FXR agonists, together with the rapid understanding that FXR controls a variety of biological and pathological processes, from BA synthesis and metabolism to cholestasis to liver regeneration, originally prompted the quest for more potent and selective FXR agonists.

A new impulse to identify FXR agonists able to treat chronic liver diseases, and NASH in particular, was provided in 2014 by the results of the FLINT trial, showing the capacity of the FXR agonist obeticholic acid (OCA) to improve histological features of NASH, including hepatic steatosis, hepatocyte ballooning, inflammation and fibrosis.[58]

Following these promising results, novel proprietary FXR agonists have been developed in an attempt to enhance FXR potency and selectivity, and to overcome at least some of the OCA-associated side effects, in particular pruritus and lipid imbalances. Several FXR agonists are currently being tested for the treatment of NASH, and key features of compounds in phase II/III clinical trials are summarized in Table 1. Compound structures are shown in Fig. 2, with compounds classified as endogenous FXR agonists, BA derivatives, non-BA-derived steroidal FXR agonists, non-steroidal FXR agonists, and partial FXR agonists.

BA-derived FXR agonists

As BAs are the natural ligands of FXR, it would seem obvious to use their scaffold to generate semi-synthetic BA derivatives with improved properties and select FXR agonists with an optimal profile. Yet, surprisingly, this strategy has been followed only by Pellicciari and coworkers. Their work led to the synthesis and characterization of 6α-ethyl CDCA (obeticholic acid, OCA), the prototypic BA-derived FXR agonist.[59]

OCA (also known as INT-747), a semisynthetic 6α-ethyl derivative of CDCA, is a first-in-class FXR agonist, originally described for its anticholestatic and hepatoprotective properties,[59] which has received accelerated approval as a second-line therapy for the treatment of primary biliary cholangitis (PBC).[60] The addition of the ethyl group to CDCA confers approximately 100-fold greater FXR agonistic activity and the structural basis for this enhanced potency (EC50 = 100 nM) has been clarified by analysis of the co-crystal structure of the FXR ligand binding domain complexed with OCA and coactivator peptide.[61] OCA also binds weakly to TGR5 (EC50 = 20 μM)[62] but it is unclear if this weak binding translates into TGR5 activation in humans.

A wealth of preclinical studies have shown the capacity of OCA to induce multiple FXR-mediated effects, including increased insulin sensitivity, hepatocyte protection against BA-induced cytotoxicity, anti-inflammatory effects in the liver, intestine and vasculature, prevention and reversal of liver fibrosis (even in animals with cirrhosis), inhibition of portal hypertension, protection of the intestinal barrier and restoration of gut microbiota eubiosis, making this drug attractive as a therapeutic agent for NASH.[13,63]

Following initial testing in patients with type 2 diabetes and NASH (NCT00501592), where a 6-week treatment with OCA increased insulin sensitivity and reduced markers of liver inflammation and fibrosis,[41] OCA was evaluated in a phase II trial (FLINT, NCT01265498) performed by the NASH-CRN, which enrolled patients with NASH without cirrhosis.[58] The primary endpoint of the trial, at least a 2-point histologic improvement in NAFLD activity score without worsening of fibrosis after 18 months of treatment, was met by 45% of patients with NASH receiving OCA 25 mg daily compared to 21% of the placebo group (p <0.001). Notably, the liver fibrosis score was significantly reduced by OCA treatment (p = 0.01). Pruritus was observed in 23% of patients receiving OCA *vs*. 6% on placebo. In contrast to the previous study, an increase in HOMA-IR (homeostatic model assessment for insulin resistance) was observed.[58] In addition, an increase in total cholesterol and LDL-C and a modest decrease in HDL-C were reported. A *post hoc* analysis subsequently showed that the increase in total cholesterol and LDL-C was largely due to an increase in less atherogenic small very low-density lipoproteins and large-buoyant LDL particles.[64] The subsequent CONTROL study showed that the addition of atorvastatin, initiated at 10 mg and titrated as needed, decreased LDL-C levels below baseline, mitigating the lipid imbalance.[65]

The phase III REGENERATE trial (NCT02548351) was conducted in patients with pre-cirrhotic NASH randomized to placebo, OCA 10 mg or 25 mg daily; an 18-month interim analysis was performed with co-primary endpoints of improvement of fibrosis by at least 1 stage without worsening of NASH and/or improvement of NASH resolution without worsening of fibrosis.[66] The first co-primary endpoint was reached by 23% of patients in the OCA 25 mg daily group compared to 12% in placebo (p = 0.0002), whereas the second one was not met. Dose dependent, rapid, and sustained reductions from baseline in liver enzymes, as well as in FIB-4 (Fibrosis-4), FibroTest,

Table 1. FXR agonists in NASH phase II/III trials.

ClinicalTrials.gov identifier acronym	Trial phase	Dose (mg/day)	Duration (wk)	Primary endpoint/s	Results	Main adverse events	Refs.	Current status
OCA (Intercept Pharmaceuticals)								
NCT00501592	II	25 50	6	Changes in insulin resistance and glucose homeostasis	Increased insulin sensitivity, reduced markers of liver inflammation and fibrosis, increased FGF19 levels	Constipation	[41]	
NCT01265498 FLINT	II	25	72	≥2-point histologic improvement in NAS without worsening of fibrosis	Primary endpoint met by 45% of patients with NASH receiving OCA *vs*. 21% of the placebo group (p <0.001)	Pruritus, increased total cholesterol, increased LDL-C, decreased HDL-C, increased HOMA-IR	[58]	
NCT02548351 REGENERATE	III	10 25	72	Proportion of treated patients relative to placebo achieving ≥1 stage of liver fibrosis improvement with no worsening of NASH and/or proportion of treated patients relative to placebo achieving NASH resolution with no worsening of liver fibrosis	Fibrosis improvement achieved by 12% of patients in placebo, 18% in the 10 mg (p = 0.045) and 23% in the 25 mg group (p = 0.0002). The NASH resolution endpoint was not met. Most secondary endpoints were met. Rapid and sustained improvements in various NITs were observed	Increased pruritus incidence (placebo 19%, 10 mg 28%, 25 mg 51%). Increased LDL-C levels reversed to below baseline levels by statin addition. Dose-dependent HDL-C decreases but within normal limits	[67,68,82,128,129]	NDA
NCT02633956 CONTROL	II	OCA 5, 10, 25 Atorv 10-20	16	Effect on LDL-C concentration and particle size. Patients received placebo or 5 mg, 10 mg or 25 mg OCA once daily for 16-weeks. Atorvastatin (10 mg/day) was initiated at week 4 with subsequent titration to 20 mg/day	At week 4, all OCA groups had increased LDL-C and LDLpc, mostly owing to large, less atherogenic LDL-C particles. Atorvastatin 10 mg decreased LDL-C and LDLpc levels below baseline in all OCA groups	Increased pruritus (placebo 5%, 5 mg 5%, 10 mg 10%, 25 mg 55%)	[65]	
EDP-305 (Enanta Pharmaceuticals)								
NCT03421431 ARGON-1	II	1 2.5	12	Mean change from baseline in ALT at week 12	No significant difference in ALT between 1 mg and placebo, marginally significant at 2.5 mg (p = 0.049). LFC not significantly different from placebo in 1 mg, significantly reduced in 2.5 mg arm (p = 0.0009)	Increased pruritus (placebo 4%, 1 mg 9%, 2.5 mg 47%)	[76]	Available for out-licensing in combination treatments
NCT04378010 ARGON-2	IIb	1.5 2	72	Same as REGENERATE	Trial discontinued after interim analysis	Not published; in QC review on Clinicaltrials.gov		
PX-104 (Phenex Pharmaceuticals AG)								
NCT01999101	II	5	4	Clinical chemistry; haematology; assessment of clinical signs and adverse events	Improved insulin sensitivity (p = 0.02); significantly reduced ALT and GGT levels	No pruritus or lipid changes. Short intervals of cardiac arrhythmia in two patients led to study termination	[79]	Discontinued
Cilofexor (Gilead Sciences)								
NCT02854605	II	30 100	24	TEAEs; treatment-emergent laboratory abnormalities	Decreased MRI-PDFF, serum GGT, C4, and primary bile acids. No changes in ELF and MRE	Increased pruritus (placebo 4%, 30 mg 4%, 100 mg 14%). No significant changes in lipids	[81]	
TERN-101 (Terns, Inc.)								
NCT04328077 LIFT	IIa	5 10 15	12	Overall safety, including TEAEs and laboratory abnormalities	Decreased ALT, GGT, MRI-PDFF, cT1 levels	Increased pruritus (placebo 0, 5 mg 16%, 10 mg 11%), 15 mg 17%. Significantly increased LDL-C and reduced HDL-C at 15 mg	[85]	Discontinued as monotherapy

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Table 1. (*continued*)

ClinicalTrials.gov identifier acronym	Trial phase	Dose (mg/day)	Duration (wk)	Primary endpoint/s	Results	Main adverse events	Refs.	Current status
Tropifexor (Novartis Pharmaceuticals)								
NCT02855164 FLIGHT-FXR	IIa/b	0.01 0.03 0.06 0.09 0.14 0.2	12/48	TEAEs; ALT; AST; liver fat content	Decreased ALT, GGT, body weight and MRI-PDFF. No effects on NAS, NASH resolution or fibrosis detected by conventional microscopy. Post hoc digital analyses revealed reductions in overall liver fibrosis and regression in perisinusoidal fibrosis	Markedly increased pruritus (placebo 22%, 140 μg 52%, 200 μg 69%). Increased LDL-C and decreased HDL-C	[88,89]	Available for partnering
MET-409 (Metacrine)								
	Ib	50 80	12	Safety and tolerability	Decreased MRI-PDFF. Transiently increased ALT levels. Decreased GGT levels	Increased pruritus (placebo 0%, 50 mg 16%, 80 mg 40%) Increased LDL-C and decreased HDL-C levels	[92]	Discontinued
Vonafexor (ENYO Pharma)								
NCT03812029 LIVIFY	IIa	100 BID 200 400	12	Change in absolute liver fat content; safety	Liver fat reduction by MRI-PDFF, reduced liver enzymes, weight loss, and a possible renal benefit based on changes in glomerular filtration rate	Increased pruritus (placebo 6%, 100 mg 61%, 200 mg 51%). Increased LDL-C and decreased HDL-C levels	[94]	
Nidufexor (Novartis Pharmaceuticals)								
NCT03804879	II	50 100	12	Ratio to baseline in UACR; ratio to baseline in 24 h urinary albumin at week 24; safety	Significant reductions in ALT levels, MRI-PDFF (p <0.0001 *vs*. placebo), body weight	Increased pruritus (placebo 15%, 50 mg 29%, 100 mg 54%). Decreased HDL-C	[104]	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BA, bile acid; C4, 7a-hydroxy-4-cholesten-3-one; cT1, corrected T1; GGT, gamma-glutamyltransferase; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein-cholesterol; LDLpc, LDL particle concentration; LFC, liver fat content; MRE, magnetic resonance elastography; MRI-PDFF, MRI-estimated proton density fat fraction; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; NDA, new drug application; NITs, non-invasive tests; OCA, obeticholic acid; TEAEs, treatment-emergent adverse events; UACR, urinary albumin-creatinine ratio.

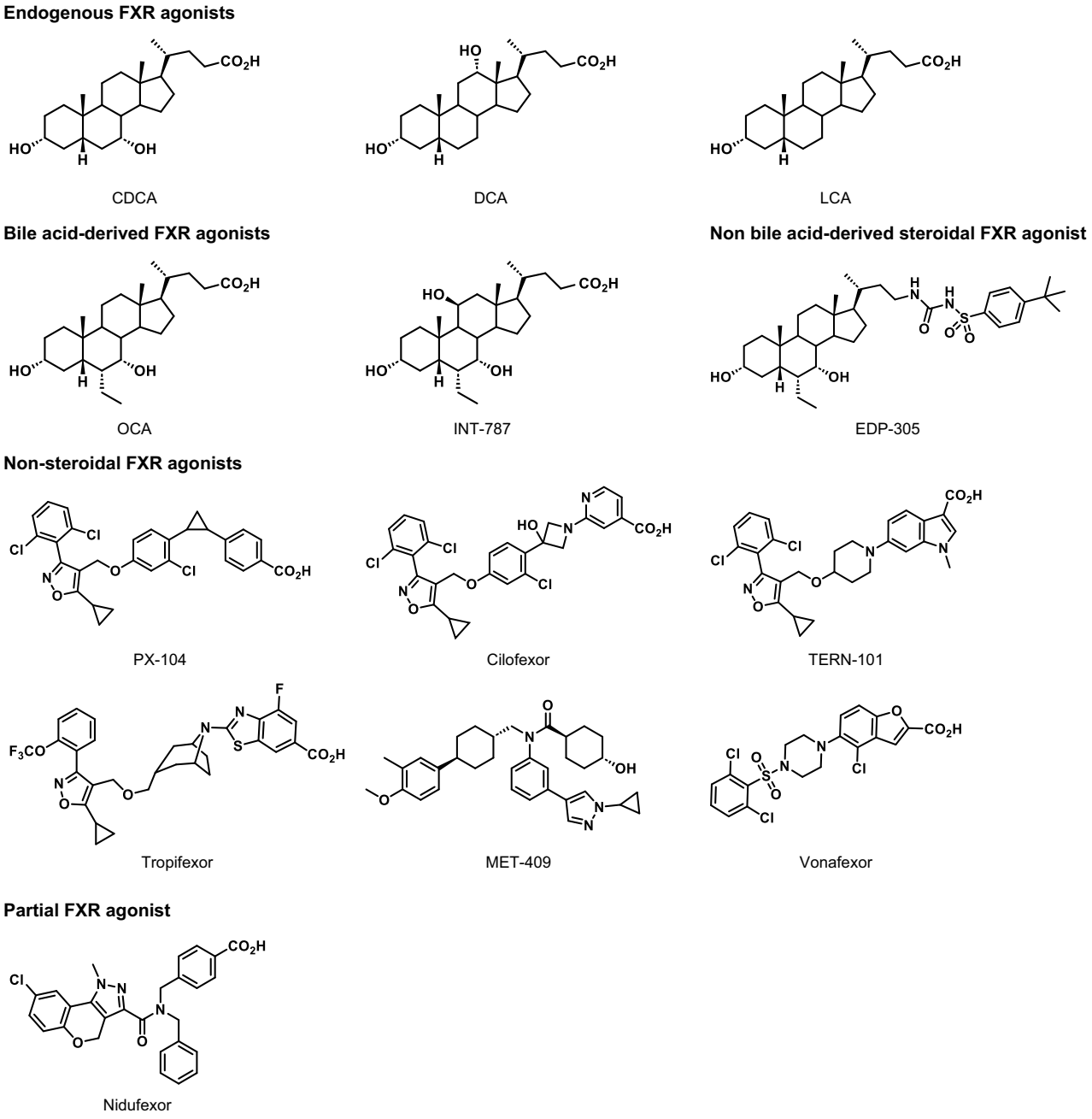

Fig. 2. Compound structures of FXR agonists. CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; FXR, farnesoid X receptor; LCA, lithocholic acid; OCA, obeticholic acid.

FibroMeter, and FibroScan-AST scores were observed in OCA-*vs.* placebo-treated patients. Reduced liver stiffness by vibration-controlled transient elastography was observed in the OCA 25 mg group *vs*. the placebo group at month 18.[67] NASH improvement was paralleled by improvement in several health-related quality of life domains.[68]

The most prevalent adverse event observed in REGENERATE was pruritus, which was present in 51% of the OCA 25 mg group and 19% of the placebo group. Pruritus was the most common reason for drug discontinuation, leading to discontinuation in 9% of the OCA 25 mg group. However, pruritus was generally mild and did not worsen over time. Hepatic serious adverse events occurred in <1% of patients across all groups. More patients within the OCA treatment groups qualified for treatment of high levels of LDL-C over the trial duration (17% *vs.* 7% in the placebo group). Hepatobiliary events (gallstones or cholecystitis) were more frequent in OCA 25 mg (3%) compared to placebo (<1%) and OCA 10 mg (1%) groups.[58] This might be explained by FGF19-triggered gallbladder relaxation in combination with increased cholesterol saturation and BA hydrophobicity in bile.[69]

The CONTROL study (NCT02633956) was designed to test the effect of the lipid-lowering statin atorvastatin on LDL-C and LDL particle concentration (LDLpc). NASH patients

received placebo or 5 mg, 10 mg or 25 mg OCA once daily for 16 weeks. Atorvastatin (10 mg/day) was initiated at week 4 with subsequent titration to 20 mg/day. At week 4, all OCA groups showed increased LDL-C and LDLpc, mostly owing to large, less atherogenic LDLc particles. Atorvastatin 10 mg decreased LDLc and LDLpc levels below baseline in all OCA groups by week 8; higher doses did not provide additional clinical benefits.[65] Thus, OCA-induced LDL-C increases in patients with NASH can be mitigated with low-dose atorvastatin.

In a complete response letter (CRL) issued in 2020, the US Food and Drug Administration (FDA) determined that the predicted benefit of OCA in NASH, based on a surrogate histopathologic endpoint, remains uncertain and does not sufficiently outweigh the potential risks to support accelerated approval, recommending submission of additional post-interim analysis of efficacy and safety data from the ongoing REGENERATE study. A new drug application (NDA) was then submitted and accepted by the FDA. Ahead of the FDA decision, the Gastrointestinal Drugs Advisory Committee reviewed information provided by the FDA and Intercept from the OCA NDA for the treatment of pre-cirrhotic fibrosis due to NASH and concluded that, given the available efficacy and safety data, the benefits of OCA 25 mg treatment do not outweigh the risks in patients with NASH and stage 2 or 3 fibrosis, citing modest drug efficacy and considerable risk of adverse events. The Committee also voted to defer approval until clinical outcome data from the REGENERATE trial are submitted and reviewed, at which time the traditional approval pathway could be considered. Based on this opinion, the FDA issued in June 2023 a new CRL deciding against accelerated approval of OCA in NASH. This negative view will likely affect the entire field of FXR agonists in NASH and will probably dampen efforts to develop novel FXR agonists for this indication.

INT-787 (also known as TC-100) is a novel OCA derivative hydroxylated at position C11β, resulting in a compound equipotent to OCA at FXR but with no agonistic activity on TGR5.[70] INT-787 has a 16-fold higher water solubility than OCA, comparable to CA, and a high critical micellar concentration comparable to ursodeoxycholic acid, a bile acid that is safely dosed at 13-15 mg/kg/day for the treatment of PBC,[71] resulting in very low liability for detergency and thus toxicity.[70] Preclinical studies in a NASH model indicate superior efficacy of INT-787 compared to OCA, based on improved liver enzyme and histologic effects and enhanced gene regulation in liver and gut (Adorini L. *et al.* manuscript in preparation). In a mouse model of bile duct ligation, INT-787 treatment activates FXR in the gut-liver axis, leading to a significant reduction of BA pool size with a shift to a more hydrophilic composition, and no signs of intestinal mucosal damage.[70,72] Verrucomicrobia, and in particular *Akkermansia muciniphila*, known for improving gut homeostasis and immune functions, are markedly enhanced by INT-787 treatment, indicating the ability of this compound to activate liver and intestinal FXR, thereby preventing gut mucosal damage by modulating BA homeostasis and microbial composition.[72] Based partly on these data and on a favourable safety and tolerability profile in an ongoing phase I study, a phase IIa trial evaluating safety, tolerability, efficacy, and pharmacokinetics of INT-787 has been initiated in patients with severe alcohol-associated hepatitis (NCT05639543).

Non-BA-derived steroidal FXR agonists

Among the few non-BA-derived steroidal FXR agonists so far developed, EDP-305 is a steroidal non-carboxylic acid compound, which lacks the carboxylic acid group required for formation of the taurine and glycine conjugates typical of BAs, and which has been modified with additional non-steroidal binding elements to enhance FXR potency (EC50 = 8 nM) while retaining FXR selectivity.[73] EDP-305 regulates multiple pathways inducing anti-lipogenic, anti-inflammatory, and anti-fibrotic gene signatures. Consistent with this *in vitro* gene regulation, EDP-305 showed hepatoprotective and anti-steatotic effects in mouse NASH models[73] and reduced fibrosis progression.[74] Interestingly, in nearly all the read-outs analysed in preclinical studies, EDP-305 appears to be more effective than OCA in reducing liver injury and fibrosis.[75]

A phase II trial showed that EDP-305 reduces alanine aminotransferase (ALT) levels and liver fat content in patients with NASH, but dose-dependently increases generalized pruritus (by up to 47% in the EDP-305 2.5 mg group).[76] Although these data were considered to provide support for a longer-term trial assessing histological endpoints, Enanta has abandoned development of EDP-305 as a monotherapy in NASH.

Non-steroidal FXR agonists

The majority of FXR agonists that have entered advanced clinical testing for NASH treatment are non-steroidal compounds. Several of them, including PX-104, cilofexor, TERN 101 and tropifexor are derived from GW4064, the first in class isoxazole-type FXR agonist, which has only been used as a research tool and could not be developed due to limited solubility, potential toxicity, UV instability and poor pharmacokinetics.[77] GW4064-derived compounds are all potent FXR agonists but in general exhibit poor aqueous solubility and bioavailability.[78] Although this is not preventing their clinical development, some compounds in this class, like PX-104 (parent compound of cilofexor), and TERN-101, have been discontinued as monotherapy for NASH treatment after initial clinical testing.

PX-104 was tested in a 4-week trial in patients with NAFLD (NCT01999101), wherein it led to improved insulin sensitivity in 92% of patients ($p = 0.02$) and significantly reduced ALT and gamma-glutamyltransferase (GGT) levels.[79] No pruritus or lipid changes were reported. Although no serious adverse events were observed, short intervals of cardiac arrhythmia in two patients led to termination of the study.[79]

Cilofexor (GS-9674), a differentiated follow-up compound of PX-104, is a selective and potent FXR agonist (EC50 = 43 nM) able to decrease portal hypertension in cirrhotic and non-cirrhotic experimental models by targeting vascular remodelling and sinusoidal dysfunction without affecting systemic haemodynamics.[53] In addition, using a cirrhotic NASH model, cilofexor was shown to significantly reduce liver fibrosis and intrahepatic sinusoidal resistance, leading to reduced portal hypertension via an apparent deactivation of HSCs and inhibition of fibrogenesis.[54] A single 100 mg dose of cilofexor showed good tolerability in healthy volunteers without major adverse effects.[80] In a phase II trial in patients with non-cirrhotic NASH, a 24-week therapy with cilofexor was safe and associated with significant reductions in hepatic steatosis,

liver biochemistry, and BA levels,[81] although the beneficial effects on liver biochemistry were modest compared to those observed with OCA over a 24-week treatment period.[58,82] Reductions in multiple serum markers of fibrosis suggest that cilofexor may have antifibrotic effects.[81] Although no significant effects were observed on serum lipids in cilofexor-treated patients, moderate to severe pruritus was more common in patients receiving cilofexor 100 mg (14%) than in those receiving cilofexor 30 mg (4%) and placebo (4%).[81]

TERN-101 (LY2562175) is a potent FXR agonist with an EC50 value of 193 nM[83] and is able to significantly reduce TGs and LDL-C and to elevate HDL-C in the insulin-resistant ZDF rat model.[83] The overall safety, pharmacokinetic and pharmacodynamic profiles of TERN-101 in healthy volunteers supported its further evaluation in patients with NASH.[84] TERN-101 was tested in a phase IIa study (NCT04328077, LIFT) in patients with clinical or histological NASH without cirrhosis randomized to placebo or TERN-101 (5, 10, or 15 mg) for 12 weeks. The primary endpoint was safety; secondary and exploratory endpoints included change in ALT, MRI-estimated proton density fat fraction (MRI-PDFF), and corrected T1 (cT1) relaxation time. Numerical reductions in ALT and MRI-PDFF and a significant reduction in GGT were observed at 10 and 15 mg doses. Significant decreases in cT1 were observed at all doses, suggesting decreased fibro-inflammation. Pruritus occurred in 11-17% of TERN-101- *vs*. 0 placebo-treated patients. Significantly increased LDL-C and decreased HDL-C were observed at the 15 mg dose.[85]

Tropifexor (LJN-452) is the most potent non-steroidal selective FXR agonist (EC50 = 0.2 nM) being developed. Tropifexor has been tested in two mouse models of NASH, wherein it led to reduced steatohepatitis, decreased hepatic TGs, reversal of established liver fibrosis and a gene expression profile suggestive of reduced oxidative stress, fibrogenesis, and inflammation. The efficacy of tropifexor at <1 mg/kg doses was superior to that of OCA at 25 mg/kg in both NASH models.[86] In a phase I study, tropifexor showed dose-dependent target engagement without altering plasma lipids in healthy volunteers.[87] Phase II data from the adaptive FLIGHT-FXR trial (NCT02855164) provide non-significant evidence for target engagement (increased FGF19 levels), anti-inflammatory (decreased ALT and GGT levels) and anti-steatotic (decreased MRI-PDFF) effects, with a slightly increased rate of pruritus, modestly increased LDL-C and reduced HDL-C following treatment with tropifexor at 60 or 90 μg/day for 12 weeks.[88] The 48-week-long part C of the FLIGHT-FXR study with tropifexor 140 or 200 μg/day showed significantly decreased ALT, GGT, body weight and MRI-PDFF but markedly increased pruritus (22% in placebo, 52% in 140 μg and 69% in 200 μg groups). No significant differences in NAFLD activity score, NASH resolution or liver fibrosis were observed between tropifexor and placebo groups.[88] Although no differences in fibrosis could be observed by conventional microscopy, *post hoc* digital analyses revealed tropifexor-associated reductions in overall liver fibrosis as well as regression in perisinusoidal fibrosis in patients with F2 or F3 fibrosis at baseline.[89]

MET-409, a non-steroidal FXR agonists different in structure from the isoxazole-type, carboxylic acid-bearing FXR agonists (Fig. 2), is based on fexaramine,[90] a potent (EC50 = 25 nM) and selective gut-restricted FXR agonist able to markedly induce FGF15, modulating BA composition and several metabolic parameters without activating FXR target genes in the liver.[91] A 12-week phase Ib study of MET-409 in patients with NASH showed marked dose-dependent liver fat reduction by MRI-PDFF, increased LDL-C and reduced HDL-C levels, and an increased proportion of patients with treatment-related pruritus (placebo 0%, MET-409 50 mg 16%, 80 mg 40%). ALT values were increased following MET-409 50 mg but decreased by the 80 mg dose.[92]

Vonafexor (EYP001), a non-steroidal, carboxylic acid FXR agonist equipotent to OCA but with no activity on TGR5, has been shown to induce FXR target gene mRNA expression *in vitro*.[93] Vonafexor significantly improved NASH parameters, including fibrosis, ballooning, and portal inflammation in a mouse model of NASH.[93] Based on these preclinical efficacy findings, and safety, tolerability, pharmacokinetics and pharmacodynamics data obtained in phase I clinical trials, vonafexor has been tested in a phase II clinical trial in patients with suspected NASH.[94] After 12 weeks of treatment, vonafexor met the primary endpoint of the trial, liver fat reduction assessed by MRI-PDFF, and led to reductions in body weight, liver enzymes, and cT1, as well as improvements in creatinine-based glomerular filtration rate.[94] Mild to moderate generalized pruritus was reported in 6%, 10%, and 18% of patients in the placebo, vonafexor 100 and vonafexor 200 mg arms, respectively.

Partial FXR agonists

Given the pleiotropic activities of FXR, partial FXR activation represents a potentially valuable strategy to avoid mechanism-based side effects of FXR targeting.[95] Partial FXR agonists behave like full agonists in weakening the FXR-ligand binding domain (LBD)-corepressor interaction, but in contrast to full agonists they induce a conformation of the FXR-LBD with affinity for both coactivators and corepressors, thus resulting in selective target gene modulation.[96] Moreover, different FXR agonists induce different receptor conformations, which can then drive co-regulator, tissue- and gene-selectivity.[97] Binding to allosteric pockets within the FXR-LBD may also lead to conformational modulation with different biological outcomes.[98] Activation of specific FXR isoforms could in principle also serve to modulate gene-selective programmes via distinct motif binding.[99] Mechanisms for gene-selective modulation following FXR activation include control of co-factor binding and subsequent FXR post-translational modifications, like O-GlcNAcylation, phosphorylation, acetylation, sumoylation, and methylation, reviewed in,[100,101] which could enhance or inhibit FXR transcriptional activity. Thus, modulation of FXR post-translational modifications offers the opportunity to target specific subsets of FXR-regulated genes.[95] As an example, FXR agonist-activated sumoylation at K277, which is necessary for transrepression of pro-inflammatory genes, is inhibited by acetylation at K217 in obese individuals and targeting this acetylation site is postulated to reduce hepatic inflammation and improve glucose tolerance.[102] However, while this wide variety of mechanisms theoretically offers multiple opportunities for selective modulation of FXR-mediated signal transduction, the current state of the art does not yet permit designed reductions to practice.

The partial FXR agonist nidufexor (LMB763) shows selective activation of FXR target genes *in vitro* and behaves as a potent and specific gene modulator *in vivo*, leading to reduced steatosis, inflammation, and fibrosis in a mouse model of NASH.[103] Nidufexor was found to be well-tolerated in healthy volunteers

and has been evaluated in a 12-week phase II clinical trial in patients with NASH and diabetic nephropathy (NCT03804879). The results showed liver fat reduction by MRI-PDFF, accompanied by reduced ALT and body weight. There were no significant changes in total cholesterol, LDL-C or triglycerides; HDL-C decreased by 12% and 16% in patients receiving 50 mg or 100 mg nidufexor, respectively. Pruritus was reported in 15%, 29%, and 54% of patients in the placebo, 50 mg and 100 mg nidufexor groups, respectively.[104]

Prospects for combining FXR agonists with other agents in NASH treatment

The pleiotropic pathogenesis of NASH offers multiple attack points for an effective treatment.[3,105] While FXR, as summarized above, can target different key pathways in NASH, combining FXR agonists with complementary agents may provide additive if not synergistic efficacy, and possibly reduce side effects.[106] This concept has been demonstrated in a NASH model, wherein the combination of OCA with the dual PPAR-α/δ agonist elafibranor was shown to exert additive effects on metabolic and histological endpoints compared to monotherapies.[107] In line with their different molecular mechanisms of action, OCA and elafibranor monotherapies elicited distinct hepatic gene expression profiles and their combination led to profound transcriptome changes associated with further improvements in lipid handling and insulin signalling, inhibition of immune responses and reduced ECM formation, providing preclinical proof of concept for this approach.[107] Combinations of FXR agonists with a variety of different agents, in particular drugs targeting glucose and lipid metabolism, are currently being extensively tested in patients with NASH.

Tropifexor has been combined with cenicriviroc, a dual antagonist of C–C chemokine receptor types 2 and 5 shown to reduce liver fibrosis in a phase II study,[108] in the 48-week, phase IIb TANDEM study (NCT03517540) in patients with biopsy-proven NASH and liver fibrosis.[109] Safety profiles of the combination therapy were comparable to those of each monotherapy. Tropifexor monotherapy showed sustained ALT and body weight decreases, but no substantial incremental efficacy was induced by combination with cenicriviroc on ALT, body weight, or histological endpoints compared with monotherapy, and there was no evidence for superiority of combination treatments in fibrosis improvement or steatohepatitis resolution. Pruritus was frequent (20-40% across groups). Increases in LDL-C and decreases in HDL-C were observed in the tropifexor monotherapy and in the combination groups.[110] Tropifexor is also being tested in combination with licogliflozin, an inhibitor of sodium-glucose cotransporters 1 and 2 that has been shown to reduce serum ALT in patients with NASH,[111] in the phase II ELIVATE study (NCT04065841), which is expected to be completed in 2023.

Cilofexor combined with the acetyl-coenzyme A carboxylase inhibitor firsocostat was well tolerated and significantly reduced steatosis, lobular inflammation, and ballooning, as well as liver transaminases, bilirubin, BAs, cytokeratin-18, enhanced liver fibrosis score, and liver stiffness by transient elastography in a 48-week phase IIb trial in patients with NASH and bridging fibrosis or cirrhosis (NCT03449446), indicating reduced NASH activity and possible antifibrotic effects.[112] Cilofexor and firsocostat were also combined with semaglutide, a glucagon-like peptide-1 receptor agonist, in a 24-week phase II trial in NASH (NCT03987074), with the combination leading to a greater reduction in liver steatosis, as measured by MRI-PDFF, as well as in liver transaminases and in non-invasive tests of fibrosis compared to semaglutide alone, with all treatments being well tolerated.[113]

MET-409 was combined with empagliflozin, a sodium-glucose cotransport-2 inhibitor, in patients with type 2 diabetes and NASH in a 12-week phase IIa trial evaluating liver fat content with MRI-PDFF (NCT04702490). All regimens had favourable safety profiles, with pruritus reported in 0-6% of patients across groups. MET409 led to similar increases in LDL-C (15%) when administered as monotherapy or in combination with empagliflozin. MRI-PDFF was reduced in both monotherapy arms and demonstrated numerically additive effects in the combination regimen. However, Metacrine has halted development of all its FXR programmes in NASH.

The 12-week phase IIa DUET trial (NCT05415722) is currently evaluating TERN-501, a thyroid hormone receptor-β agonist, as a monotherapy and in combination with the FXR agonist TERN-101 in patients with NASH. The primary endpoint is the relative change from baseline in liver fat content measured by MRI-PDFF for TERN-501 monotherapy compared with placebo. Secondary endpoints include assessment of safety and tolerability, pharmacokinetics, changes in MRI-PDFF and cT1. DUET is the first trial assessing a thyroid hormone receptor-β agonist combined with an FXR agonist in patients with NASH. Top-line data are expected in the third quarter of 2023.

These results, despite not meeting expectations so far, nevertheless suggest that FXR agonists may have the potential to become a component of drug combinations aimed at targeting different pathogenetic pathways in NASH.

A global assessment of FXR agonists in NASH

Identifying ideal FXR agonists in NASH has proven to be quite challenging. While FXR potency and selectivity, as well as sound pharmacokinetic and pharmacodynamic properties, have been achieved, how to engage FXR to promote selective modulation of FXR target genes able to induce beneficial effects and minimize adverse events remains an open question. The complexity of FXR itself and of the multiple downstream pathways it activates has stymied the design of FXR agonists with all the desired properties.

The positive clinical data on OCA in NASH promoted interest in FXR agonists, and follow-up compounds were designed with two main aims in mind: improve the potency of FXR activation and reduce side effects. However, high FXR potency does not ensure *per se* a successful compound; the critical factor is the therapeutic window afforded by the balance of efficacy and side effects.

The most frequent side effects of treatment with FXR agonists in NASH are pruritus and LDL/HDL imbalances, which could potentially lead to adverse cardiovascular effects, although so far the incidence of cardiovascular adverse events in the REGENERATE cohort has been low and similar across treatment groups.[82]

Induction of pruritus has been observed in all clinical trials testing FXR agonists in NASH, including OCA, EDP-305, PX-

104, cilofexor, TERN-101, tropifexor, MET-409, vonafexor and nidufexor.[114] Therefore, induction of pruritus appears to be an FXR-mediated class effect, irrespective of the chemical structure of FXR agonists. Pruritus is a common symptom in patients with cholestatic liver disease, particularly intrahepatic cholestasis of pregnancy and PBC, but it also occurs in several other hepatobiliary diseases, including NASH.[115] The pathogenesis of pruritus in liver diseases is still unknown, although several potential players have been implicated, including BAs,[116] endogenous opioids,[117] lysophosphatidic acid,[118] steroid metabolites,[119] bilirubin,[120] and FXR-induced interleukin 31.[121] However, the contribution of these candidate pruritogens to the pathogenesis of pruritus is still unclear.[115] Thus, it is uncertain why FXR activation may induce pruritus in NASH, how FXR agonists could potentially be differentiated in this respect, and how to mechanistically approach this side effect.

An FXR-mediated class effect also appears to involve HDL/LDL imbalances: essentially all FXR agonists tested in patients with NASH have been associated with enhanced LDL-C levels, with OCA showing a modest effect in this respect,[114] and reduced HDL-C levels. In healthy volunteers, OCA induced a sustained elevation of serum LDL-C concentration (+22%) and reduction of HDL-C (-16%), with a small elevation of total cholesterol.[122] A similar profile was noted in patients with type 2 diabetes[41] and NASH.[58] As conventional animal models fail to reproduce the lipid effects observed in clinical studies, a chimeric mouse model with a humanized liver, faithfully modelling the lipid effects of FXR activation in humans, was used to compare the effects of OCA, tropifexor and cilofexor on lipid imbalances.[123] All three FXR agonists showed comparable activities on enzymes involved in cholesterol metabolism and led to similarly increased LDL-C and reduced HDL-C levels, indicating a class effect of FXR activation, acting both directly on hepatocytes and indirectly via FGF-19 induction.[123] Interestingly, the CONTROL study showed that OCA-induced increases in LDL-C in patients with NASH were due to large, less atherogenic particles, which could be reduced below baseline by concomitant treatment with atorvastatin,[65] thus providing a manageable approach to control the lipid imbalances associated with FXR agonist treatment.

A third class effect elicited by FXR agonists is the statistically significant stable increase in serum alkaline phosphatase (ALP), about a 15-20% mean increase above placebo, which has been observed in essentially all trials so far performed with FXR agonists in patients with NASH, though it is usually neglected.[41,58,76,81,82,88,92,94] ALP is an enzyme anchored to the cell membrane by glycosyl-phosphatidylinositol, which can be cleaved by phospholipase D (PLD), thus releasing ALP into the plasma. Preliminary experiments performed in human sandwich-cultured hepatocytes show that OCA does not affect *ALP* mRNA expression but dose-dependently upregulates mRNA levels of *PLD*,[124] suggesting that FXR activation does not enhance ALP transcription but leads to increased serum ALP levels via upregulation of PLD expression. The clinical significance of this finding, if any, and the source of ALP (*e.g.*, hepatic) is unknown, but it could partially obscure the marked ALP decrease induced by OCA in patients with PBC.[60]

Obviously, side effects directly mediated by FXR activation, irrespective of the specific FXR agonist tested, are difficult, if not impossible, to eliminate. Targeted FXR modulation to induce tissue-specific regulation may contribute to mitigate this problem but information regarding tissue-specific and cell-specific FXR agonists are currently lacking.[125]

If the main side effects elicited by structurally diverse FXR agonists are similar, they could still be differentiated based on efficacy. However, efficacy does not depend on potency at FXR alone but rather on a combination of physicochemical properties of the compound (*e.g.*, solubility, hydrophilicity, critical micellar concentration), mode of FXR engagement and ensuing modulation of target gene transcription, pharmacokinetics, and distribution to target organs, particularly the liver and intestine. Preferential targeting of intestinal rather than hepatic FXR has been considered as a potential strategy to widen the therapeutic window of FXR agonists by minimizing side effects and maximizing efficacy. Unfortunately, this strategy has not met expectations, as shown by the high rate of pruritus and imbalanced lipid profiles induced by the gut-targeting FXR agonist MET-409.[92]

Considering the positive clinical results induced by OCA compared to other FXR agonists, a key factor differentiating FXR agonists in terms of efficacy appears to be their capacity to enter the enterohepatic circulation. This permits compound recycling between liver and intestine, the main FXR-expressing organs targeted by FXR agonists in NASH. A long debate has argued whether NASH treatment would benefit more by targeting hepatic *vs*. intestinal FXR, concluding that targeting both may be preferable.[114,126] Among all the clinically tested FXR agonists, only BA derivatives and their glyco- and tauro-conjugates enter and are maintained within the enterohepatic circulation, ensuring sustained activity selectively in the key target organs. However, some synthetic FXR agonists can also be tauro- or glyco-conjugated *in vivo* in mice,[127] and may thus be subject to enterohepatic cycling. Enterohepatic cycling likely represents an important factor in the efficacy of FXR agonists and the exquisite ability of BA derivatives to recycle between liver and gut may contribute to explain the superiority of OCA in NASH treatment.

In conclusion, FXR agonists, and in particular BA-derived agonists, have demonstrated antifibrotic activity in NASH trials, but the concerns raised during the FDA Advisory Committee for OCA in NASH represent a major hurdle to their development as monotherapy in NASH treatment. Conversely, FXR agonists could potentially play a role in the NASH armamentarium in combination with other agents. Thus, when other therapeutic agents will be eventually approved for NASH, FXR agonists, likely at lower doses, may yet find a role in NASH treatment.

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Abbreviations

ALP, alkaline phosphatase; ALT alanine aminotransferase; BA, bile acid; cT1, corrected T1; ECM, extracellular matrix; FA, fatty acid; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GGT, gamma-glutamyltransferase; GVB, gut vascular barrier; HDL-C, high-density lipoprotein-cholesterol; HSCs, hepatic stellate cells; LBD, ligand binding domain; LDL-C, low-density lipoprotein-cholesterol; LDLpc, LDL particle concentration; LFC, liver fat content; MRI-PDFF, MRI-estimated proton density fat fraction; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; OCA, obeticholic acid; PBC, primary biliary cholangitis; PLD, phospholipase D; SHP, small heterodimer partner; SREBP-1c, sterol regulatory element factor binding protein-1c; TGR5, Takeda G protein-coupled receptor 5; TG, triglyceride.

Financial support

No funding has been provided to write this review.

Conflict of interest

LA is a consultant and shareholder of Intercept Pharmaceuticals. MT received speaker fees from BMS, Falk Foundation, Gilead, Intercept, Janssen, MSD and Roche; he advised for Abbvie, Albireo, BiomX, Boehringer Ingelheim, Falk Pharma GmbH, Genfit, Gilead, Hightide, Intercept, Janssen, MSD, Novartis, Phenex, Pliant, Regulus, Siemens and Shire. He further received travel grants from Abbvie, Falk, Gilead, Intercept and Janssen and research grants from Albireo, Alnylam, Cymabay, Falk, Gilead, Intercept, MSD, Takeda and UltraGenyx. He is also co-inventor of patents on the medical use of norUDCA filed by the Medical Universities of Graz and Vienna.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

LA wrote the first draft of the manuscript and MT provided critical intellectual input. Both authors read and edited the final version of the manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2023.07.034.

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